Western blot

Introduction

This protocol is for Western blotting you proteins.

Materials

- Transfer buffer, 20% MeOH in SDS-PAGE running buffer, 100 mL
  - 20 mL of 5x SDS-PAGE running buffer
  - 20 mL MeOH
  - 60 mL H2O
- Washing solution, PBS-T, 1 L
  - PBS with 0.05 % Tween-20
- Blocking solution, 3 % BSA or 5 % milk in PBS-T, 15 mL
  - 0,45 g BSA or 0,75 g milk powder
  - 15 mL PBS-T
- Primary antibody, 15 mL
  - 1 : 5 000 dilution of the primary antibody, 3 ul in 15 mL
  - In 3 % BSA in PBS-T or 5 % milk in PBS-T, depending on the antibody
  - Primary antibody can be used again about 10 times, add small amount of NaN3 and store in +4 °C
- Secondary antibody, 15 mL
  - 1 : 30 000 dilution of the antibody, 0.5 ul in 15 mL
  - in 1 % milk in PBS-T

Procedure

Blotting

1. Run your protein samples on SDS-PAGE as usual
2. Soak 6 pieces of Whatman blotting paper, nitrocellulose membrane (all a bit bigger than your gel) and your SDS-PAGE gel in transfer buffer for a few minutes
3. Assemble the blot: 3 layers of Whatman paper, nitorcellulose membrane (to the positive pole of the device; in the Bio-Rad blotting machine to the bottom), SDS-PAGE gel, 3 layers of Whatman paper
4. Roll out all possible air bubbles with a tube or some other device
5. Start blotting: with the Bio-Rad machine either 30 min programme, or TURBO (TURBO might not be always the best option as not all proteins will transfer)

Antibody treatment

6. **Blocking:** Put nitrocellulose membrane in to box and add blocking solution, let it incubate 1,5 h, RT, at shaking
7. **Primary antibody:** Add primary antibody solution, incubation recommended O/N, shaking, +4 °C

Can also be done at RT for 1-2 h, but less specific binding and some antibodies might degrade at RT
Collect primary antibody solution, it can be used about 10 times, add 1 % NaN3 and store in +4 °C

8. **Washing:** Rinse twice with PBS-T, and wash with PBS-T 5 min at shaking three times
9. **Secondary antibody:** Add secondary antibody solution, incubate 1 h, RT, shaking
10. Washing as earlier

Imaging

11. Mix chemiluminesence detection solutions 1 + 1 mL
12. Place the membrane on a plastic sheet and pipet the detection solution on the membrane
13. Fold the plastic and let the solution react atleast 1 min
14. Within the next hour, image with Fujifilm LAS 3000 imager. A 10 sec exposure x 3 should give good images

    Remember to take a digitized version without moving the membrane, so you can easily have the molecular weight standard in your image. Exposure time here should be 1/60 sec or 1/100 sec.